REMARKS

Applicants thank the Examiner for his review of the instant application. For the reasons stated below, the rejections of the presently pending claims are respectfully traversed. Claims 6-8 and 11-17 are presented for examination.

Rejection Under 35 U.S.C. §101

The PTO maintains its rejection of Claims 6-8 and 11-17 under 35 U.S.C. § 101 as lacking a specific and substantial utility. In particular, the PTO argues that "further research would need to be performed to place this finding into a meaningful a meaningful real world context for the encoded protein and specific antibodies to such, because without further experimentation, it cannot be determined from the encoding mRNA level if the protein level is changing in any correlated fashion with the mRNA level." *Office Action* at 4-5.

Applicants incorporate by reference their previously submitted arguments, and for the reasons of record assert that the specification contains a disclosure of utility and therefore must be taken as sufficient to satisfy the utility requirement of 35 U.S.C. § 101. Applicants also submit that for reasons of record, the PTO has not met its burden of providing evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility. However, even if the PTO has met its initial burden, Applicants' previously submitted rebuttal evidence and additional evidence submitted herewith is sufficient to prove that it is **more likely than not** that a person of skill in the art would be convinced, **to a reasonable probability,** that the asserted utility is true. As stated previously, Applicants' evidence need not be direct evidence, so long as there is a reasonable correlation between the evidence and the asserted utility. **The standard is not absolute certainty.**

Substantial Utility

Summary of Applicants' Arguments and the PTO's Response

Applicants remind the PTO that the asserted utility rests on the following argument:

1. Applicants have provided reliable evidence that mRNA for the PRO3566 polypeptide is expressed at least two-fold higher in normal skin and esophageal tumor tissue as compared to melanoma and normal esophageal tissue, respectively;

2. Applicants assert that it is well-established in the art that differential levels of mRNA for a particular protein, *e.g.* decreased levels, generally leads to corresponding differential levels of the encoded protein, *e.g.* decreased levels; and

3. Given the differential expression of the PRO3566 mRNA in melanoma and esophageal tumors as compared to their normal tissue counterparts, it is more likely than not that the PRO3566 polypeptide is also differentially expressed in melanoma and esophageal tumors as compared to their normal tissue counterparts, making the claimed polypeptides useful as diagnostic tools, alone or in combination with other diagnostic tools.

Applicants understand the PTO to be making the following arguments in response to Applicants' asserted utility:

The PTO attempts to rebut Applicants' previous arguments in view of various literature references. The PTO argues that Chen *et al.* (Mol. & Cell. Proteomics, (2002) 1:304-313) and Haynes *et al.* (Electrophoresis, (1998) 19(11):1862-71), as well as newly cited references by Nagaraja *et al.* (Oncogene, (2006) 25:2328-38), Waghray *et al.* (Proteomics, (2001) 1:1327-38) and Sagynaliev *et al.* (Proteomics, (2005) 5:3066-78) support its position that changes in the level of mRNA do not necessarily reflect changes in protein expression levels. The PTO argues that the Nagaraja *et al.*, Waghray *et al.* and Sagynaliev *et al.* references support the idea that "changes in mRNA expression frequently [do] not result in changes in protein expression." *Office Action* at 15 (emphasis in original). The PTO relies upon additional literature to support the argument that polypeptide levels cannot be accurately predicted from mRNA levels. The PTO references Lilley *et al.* ("Proteomics" Molecular Biology in Cellular Pathology, page 351 (2003)), Wildsmith *et al.* ("Gene Expression Analysis Using Microarrays," Molecular Biology in Cellular Pathology, pages 269-286 (2003)), King *et al.* (*JAMA*, 286:2280-2288 (2001)), Haynes *et al.* (see above, at pages 1863 and 1870), Bork *et al.* (Genome Res 398-400 (2000)), and Madoz-Gurpide *et al.* ("Molecular analysis of cancer using DNA and protein microarrays," *Adv. Exp. Med. Biol.*, 532:51-58).

The PTO further states that Applicants' previously submitted references are not persuasive with regard to the instant application and the question of whether differential mRNA levels correlate with differential protein levels. Also, according to the PTO, the previously submitted declarations are not persuasive.

The PTO refers to Celis *et al.* to emphasize that "proteins are frequently the functional molecules and, therefore, the most likely to reflect differences in gene expression." The PTO further states that Celis *et al.* explain that "some messengers are transcribed but not translated, and the number of mRNA copies does not necessarily reflect the number of functional protein molecules." The PTO also argues that additional information is necessary to demonstrate a utility for the claimed subject matter. In support the PTO relies upon Lee *et al.* in support of the "importance of replication in microarray gene expression studies," and relies upon King *et al.* as supporting the argument that replication in microarrays is important due the high variability in microarray expression studies. Thus, the PTO argues that the state of the art supports the assertion that nucleotide levels cannot accurately predict protein levels and that analysis of protein expression is required to identify a protein as a potential marker for cancer.

As set forth below, in light of all of the evidence, Applicants submit that the PTO's evidence cannot support the utility rejection of the claimed invention under 35 U.S.C. § 101.

The PTO has Concluded that the data in Example 18 are Sufficient to Establish the Utility of the Claimed Invention

As an initial matter, Applicants gratefully acknowledge that the PTO is persuaded by the evidence of record that when using RT-PCR, 2 fold differences in nucleic acid level can be meaningfully and reliably measured. In fact, as noted in the Office Action, the PTO has previously allowed a related patent with claims to nucleic acid sequences that encode, *inter alia*, PRO3566 polypeptides.

Also, Applicants wish to point out that in other applications filed by Applicants that rely on *data from the exact same disclosure, Example 18*, and in which the Applicants have submitted *substantially the same references* in support of their asserted utility, the PTO has concluded that:

> [b]ased on the totality of evidence of record, **one of skill in the art would find it more likely than not that an increase in message as measured by RT-PCR would be predictive of an increase in protein expression levels,** absent evidence to the contrary. Therefore, the data presented in Example 18, which demonstrates differential expression of nucleic acids encoding PRO1180, also supports a conclusion of differential expression of PRO1180 polypeptide. Therefore, one of ordinary skill in the art would be able to use the PRO1180 polypeptide diagnostically for distinguishing normal kidney and rectal tumor tissues compared to kidney tumor and normal rectal tissue, as asserted by Applicant.

See *Examiners Reasons for Allowance* in pending Application No. 10/063,529. See also *Examiners Reasons for Allowance* in Application No. 10/063,530, No. 10/063,524, No. 10/063,582, and No. 10/063,583, all of which conclude that the data presented in Example 18, which demonstrate differential expression of the nucleic acids encoding certain PRO polypeptides, also support a conclusion of differential expression of the PRO polypeptides, making the claimed PRO polypeptides and antibodies that bind the PRO polypeptides useful for diagnostic purposes. These are just a few examples from many cases where the PTO has agreed with Applicants' position as set forth in their co-pending applications and allowed polypeptide and antibody claims.

Applicants therefore request that the Examiner recognize the utility of the claimed invention, supported by the data presented in Example 18 and the numerous cited references, as was done in the other applications, including those referenced above.

Applicants Specification Discloses Differential Expression of PRO3566 mRNA between Normal Skin Tissue and Melanoma, and Between Esophageal Tumor and Normal Esophageal Tissue

Example 18 of the Specification teaches that mRNA encoding the PRO3566 polypeptide is differentially expressed in normal skin and esophageal tumor tissue as compared to melanoma and normal esophageal tissue, respectively. Given the differential expression of the PRO3566 mRNA in melanoma and esophageal tumors as compared to their normal tissue counterparts, it is more likely than not that the PRO3566 polypeptide is also differentially expressed in melanoma and esophageal tumors as compared to their normal tissue counterparts, making the claimed polypeptides useful as diagnostic tools, alone or in combination with other diagnostic tools.

Chen et al.

The Office Action continues to cite Chen *et al.* in asserting that differential mRNA expression levels do not lead to corresponding differential levels of the encoded polypeptides. Applicants incorporate by reference their previously submitted arguments in regard to Chen *et al.* and will not completely reiterate those arguments here.

The PTO acknowledges that Figures 2A-2C of the Chen reference represent three samples where protein levels correlated well with mRNA. However, the PTO argues that Chen *et al.*

"also reported 137 protein spots wherein protein levels did not correlate with mRNA levels." The PTO concludes that Chen *et al.*, teaches that:

> mRNA levels are not predictive of protein levels, stating '[t]he use of mRNA expression patterns by themselves, however, is insufficient for understanding the expression of protein products, as additional post transcriptional mechanisms, including protein translation, post-translational modification, and degradation, may influence the level of a protein present in a given cell or tissue.'

Applicants respectfully disagree with the PTO's argument. Chen *et al.* attempted to discover a global ratio, *i.e.*, a single numerical ratio, which is common for all steady state mRNA levels and all steady state protein levels. A high global relationship according to Chen *et al.* would refer to there being a "highly" consistent single numerical ratio of the level of every polypeptide in a cell relative to the level of the corresponding mRNA. Whether such a single numerical ratio is highly consistent or not is irrelevant to Applicants' asserted utility.

That is, whether or not a single numerical ratio exists which is common for all steady state mRNA levels and all steady state protein levels is irrelevant to Applicants' asserted utility. Applicants assert that differential mRNA levels for a particular gene typically lead to corresponding differential levels for the encoded protein. Contrary to the PTO, Chen *et al.* cannot be said to stand for the proposition that mRNA levels are not predictive of protein levels for differentially expressed molecules. Thus, there are simply no data in the reference that would serve to counter Applicants' claims regarding differential mRNA and polypeptide levels because there are no data of differential mRNA or polypeptide levels in the reference.

Whether or not mRNA levels predict absolute protein levels is simply not relevant to Applicants' asserted utility. As explained in the first Grimaldi Declaration:

> The results of the gene expression studies indicate that the genes of interest can be used to differentiate tumor from normal. The precise levels of gene expression are irrelevant; what matters is that there is a relative difference in expression between normal tissue and tumor tissue. *First Grimladi Declaration* at paragraph 7 (emphasis added).

Applicants' asserted utility does not require that precise protein levels can be predicted. Instead, Applicants assert that the report in Example 18 of relative difference in mRNA expression between normal tissue and tumor tissue is sufficient to establish the nucleotide encoding the PRO3566 polypeptide as useful for differentiating tumor from normal. The PTO

does not argue to the contrary. The PTO appears only to contest Applicants' assertion that differential levels of mRNA for a particular protein, *e.g.*, decreased levels, generally leads to corresponding differential levels of the encoded protein, *e.g.*, decreased levels. Therefore, the only issue which remains is whether the data in Example 18 regarding differential expression of the PRO3566 mRNA are reasonably correlated with differential expression of the PRO3566 polypeptide such that the claimed polypeptides have utility as diagnostic tools as well. As discussed below, PTO's evidence is insufficient to establish a reasonable doubt regarding Applicants' assertion that they are reasonably correlated in view of Applicants' overwhelming rebuttal evidence that differential mRNA levels lead to corresponding differential protein levels.

Previously Submitted Declarations and Exhibits with Numerous Literature References Are Relevant to and Rebut the PTO's Argument Against Allowance of the Claims

Applicants previously submitted Exhibits 3-22, comprising 113 references, in support of their argument for the correlation between mRNA levels and protein levels. Applicants have previously submitted many other references in support of their position. Despite Applicants' several declarations to the contrary; world-renowned, peer reviewed textbooks to the contrary; numerous articles supporting Applicants position; and other Examiners at the PTO agreeing with Applicants' position on utility and allowing cases with polypeptide claims based upon the data in Example 18; the Examiner continues to conclude in this case that there is no utility.

The PTO discounts the numerous studies submitted by Applicants. The PTO argues that "all of Applicant's newly cited references, with the exception of Futcher et al., are directed the analysis of single genes, or a small group of genes, and therefore do not demonstrate trends found across proteins in general." The PTO also discounts Orntoft *et al.* In response to Applicants' assertion, the PTO newly cites references by Nagaraja *et al.* (Oncogene, (2006) 25:2328-38); Waghray *et al.* (Proteomics, (2001) 1:1327-38); and Sagynaliev *et al.* (Proteomics, (2005) 5:3066-78); as well as previously cited Chen *et al.* and Haynes *et al.*, arguing that these references are more persuasive since they disclosed "comprehensive studies where significantly large numbers of transcripts and proteins were examined and more accurately describe general trends." Applicants respectfully disagree.

Applicants again remind the PTO of the correct standard for satisfying the utility requirement; whether it is more likely than not that a person of skill in the art would be convinced, to a reasonable probability, that the asserted utility is true. As stated previously, Applicants' evidence need not be direct evidence, so long as there is a reasonable correlation between the evidence and the asserted utility. The standard is not absolute certainty as suggested by the PTO.

Applicants assert that it is well-established in the art that differential levels of mRNA encoding a particular protein generally lead to a corresponding difference in the level of the encoded protein; given Applicants' evidence of differential expression of the mRNA for the PRO3566 polypeptide normal skin and esophageal tumor tissue as compared to melanoma and normal esophageal tissue, respectively, it is likely that the PRO3566 polypeptide is also differentially expressed; and proteins differentially expressed in certain tumors, and antibodies that bind such proteins, have utility as diagnostic tools.

Applicants have already discussed and explained the irrelevance of Chen *et al.* and Haynes *et al.* Furthermore, as Applicants explain below, review of the data and findings of the other references demonstrates that these references provide only a small amount of data relating to both mRNA and protein levels, and almost none of the data and conclusions of these references are contrary to Applicants asserted utility.

The PTO argues that in Nagaraja *et al.*, researchers observed that there were fewer changes observed in protein abundance as compared to transcript abundance between various malignant and normal breast cell lines and that "'[t]he comparison of transcript profiles with proteomic profiles demonstrated that altered proteins were not always represented in the microarray designated profiles and *vice versa*.'" *Id.* at 9. The PTO sees these observations as support for its contention that differential mRNA levels are not predictive of differential protein levels. However, careful examination of Nagaraja *et al.* shows that the reference does not contain evidence that supports the PTO's position.

Nothing in Nagaraja's results would suggest that differentially expressed mRNA do not typically have a corresponding differentially expressed encoded polypeptide. Nagaraja does not list how many differential mRNA levels and encoded polypeptide levels did or did not correspond to each other. All Nagaraja teaches is that "altered proteins were not always

represented in the microarray designated profiles and *vice versa*." *Nagaraja* at 2329, left column. Nagaraja does not state that in all or even most instances, differential mRNA levels and encoded polypeptide levels did not correspond to each other. It is simply not known how many mRNA/protein pairs were examined, and what percentage of those showed a correlation between differential mRNA levels and protein levels. Nothing in this statement would lead one to conclude that differential mRNA levels do not typically correspond to differential levels of the encoded polypeptide.

Furthermore, Nagaraja's experimental methods and results are not contrary to Applicants' assertions. Nagaraja's "comprehensive study" looked at no more than 25 mRNA/protein pairs. The total number of proteins detected in the most sensitive protein gel was about 300, which was less than 2% of the total number of transcripts that were identified by Nagaraja's microarray analysis. The proteins selected by Nagaraja as differentially expressed were only those proteins that were solely detected in the malignant cell lines, as compared to the normal cell line. Proteins that were detected in the normal cell line, whether upregulated, downregulated, or absent in the malignant cell lines relative to the normal cells lines, were omitted from the proteosome analysis. In the end, only 25 proteins were examined, which is about 2.5% of the number of differentially expressed transcripts detected. Even then, Nagaraja does not indicate whether or not corresponding transcripts were detected for each of these 25 proteins. Thus, Nagaraja looked at 25 or fewer mRNA/protein pairs.

Nagaraja's experimental methods and results do not teach even a single instance in which differentially expressed mRNA did not have a similarly differentially expressed encoded polypeptide. Nagaraja may very well have detected some differentially expressed proteins that were not associated with differentially expressed mRNAs. Such a result is not contrary to Applicants' assertions that differential mRNA levels typically correspond to differential levels of the encoded polypeptide. Evidence in Nagaraja of differentially expressed protein when mRNA is not differentially expressed cannot lead to the conclusion that differential mRNA levels fail to lead to corresponding differential protein levels. Accordingly, these results cannot be construed as contrary to Applicants' assertions.

The "*vice versa*" side of Nagaraja's statement that "altered proteins were not always represented in the microarray designated profiles and *vice versa*" reflects nothing more than

Nagaraja's results based on the methods used. Nagaraja observed over 1000 differentially expressed transcripts out of as many as 18,400 measured, but only identified 25 of about 300 total measured proteins as present in tumor cell lines but not in normal cell lines. Thus, the vast majority of polypeptides encoded by differentially expressed mRNA were not even measurable by Nagaraja's methods, much less measured as missing in normal cell lines and present in tumor cell lines. Additionally, over 90% of the 300 or so detectable proteins were eliminated from consideration, differentially expressed or not, because they were detected at any level in the normal cell line. Thus, it is a necessary conclusion based on Nagaraja's methods that the over 1000 differentially expressed mRNAs would not always be represented in the set of 25 proteins found as expressed in tumor cell lines and not in the normal cell line simply because Nagaraja's methods were only able to detect a few proteins and the method for selecting the 25 proteins removed all differentially expressed proteins that were present in the normal cell line. Any suggestion by Nagaraja that mRNA and polypeptide levels are not related is merely a reflection of the disparate sensitivities of Nagaraja's methods for measuring nucleotides versus proteins and the criterion used for selecting the 25 proteins. Accordingly, these results cannot be construed as contrary to Applicants' assertions.

In sum, Nagaraja's statements, even if taken out of context, merely lead to the conclusion that differential mRNA levels do not always correspond to differential protein levels; nothing in the reference suggests that differentially expressed mRNA do not typically have a corresponding differentially expressed encoded polypeptide. Furthermore, nothing in Nagaraja's experimental methods and 25 results reflect even a single instance in which differentially expressed mRNA did not have a similarly differentially expressed encoded polypeptide. Therefore, Nagaraja cannot serve as evidence contrary to Applicants' asserted utility.

The PTO cites Waghray *et al.* as teaching that, "for most of the proteins identified, there was no appreciable concordant change at the RNA level." The PTO also cites Waghray *et al.* as stating that the "change in intensity for most of the affected proteins identified could not be predicted based on the level of the corresponding RNA."

However, Applicants again repeat that they make no assertion that differential protein levels always are accompanied by differential mRNA levels. The possibility that additional factors beyond differential mRNA levels also can lead to differential protein levels does not

imply that differentially expressed mRNA do not typically have a corresponding differentially expressed encoded polypeptide.

Waghray's "comprehensive study" looked at 29 mRNA/protein pairs. Waghray *et al.* looked at transcriptosomal and proteosomal changes in an androgen-sensitive prostate cancer cell line after the cells were treated with dihydrotestosterone (DHT). Out of 16,570 genes, the authors found 351 transcripts that were differentially expressed in the stimulated cells. *Waghray* at 1329, right column. The authors identified 44 proteins, out of 1031 spots on protein gels that were upregulated or downregulated in stimulated cells. *Waghray* at 1333, right column. Of these 44 proteins, 29 were identified without ambiguity. *Id.* Of these 29 identified proteins, only 7 had a corresponding mRNA that changed by at least 2-fold as measured by the SAGE method. *Waghray* at 1336 (Table 4). Applicants' repeat that they make no assertion that differential protein levels must lead to similarly differential mRNA levels. Therefore, only these 7 instances that report differential mRNA levels bear relevance to Applicants' assertions.

Of these 7, five had values close to zero. Waghray teaches that when the value detected using SAGE "is close to zero, the quantitative nature of SAGE is compromised." *Waghray* at 1337, left column. Further, Waghray provides examples that when the differential SAGE levels were both close to zero, the levels measured by the more accurate PCR method showed only a small (<1.5-fold) difference. *Waghray* at 1337, left column, and Figure 1D. Thus in Waghray's "comprehensive study," only 2 discernibly differentially expressed mRNAs also had measurements of the corresponding proteins. Applicants concede that in these 2 instances, the differential mRNA levels reported in Table 4 were not accompanied by similarly differential protein levels. However, this "comprehensive study" of 2 differentially expressed mRNAs falls far short of serving as demonstrative evidence that Applicants' 115 abstracts fail to establish that typically, differential mRNA levels are accompanied by corresponding differential levels of the encoded protein.

Even these 2 instances may be artifacts of the mRNA and protein measurements made at different time points in Waghray's methods. The authors noted that the dynamic conditions of the experiments created fluctuating levels of both mRNA over time and that each of three transcripts displayed different dynamic behavior. *Waghray* at Fig. 1C and 1337. But Table 4 reports only 24 hour time points for each transcript, without providing a basis for selection of this

arbitrary timepoint. None of the mRNA and protein data from Table 4, upon which the authors base their observation about the concordance of mRNA and protein levels, address the dynamic fluctuation taught by Waghray. Thus, Waghray teaches dynamic fluctuation of mRNA levels and the arbitrary timepoint selected to detect mRNA levels to be compared to protein levels, regardless of degree of fluctuation of these levels. Further, Waghray provides no basis for presuming that the reported protein levels do not also exhibit the same variability due to the arbitrary 72 hour timepoint selected for measurement. More importantly, there is no basis in Waghray to consider that the 24 hour timepoint for mRNA levels is appropriate for comparing to the 72 hour timepoint for protein levels. Since both mRNA levels and protein levels can be expected to be in dynamic fluctuation, there is no basis in Waghray for concluding that the data in Table 4 should accurately reflect the relationship between mRNA and protein levels in the cell.

In sum, Waghray presents no more than 2 discernibly differentially expressed mRNAs for which polypeptide levels were not similarly differential. However, based on Waghray's arbitrarily selected timepoints for measurement, there is no way to conclude that these 2 instances accurately reflect the relationship between mRNA and protein levels in the cell. As such, Waghray provides little or no basis to doubt Applicants' asserted utility.

The PTO also cites the work of Sagynaliev *et al.* to support its argument that mRNA levels are not predictive of protein levels, even when considering changes in mRNA levels. The PTO quotes from Sagynaliev: "It is also difficult to reproduce transcriptomics results with proteomics tools. Out of 982 genes found to be differentially expressed in human CRC by genome-wide transcriptomics technologies (Table 6a), only 177 (18%) have been confirmed using proteomics technologies."

The work of Sagynaliev *et al.*, however, is not contrary to Applicants asserted utility. The Sagynaliev *et al.* reference is a review of scientific papers regarding gene expression in colorectal cancer (CRC) and attempts to create a "data warehouse" by combining the results of multiple researchers and laboratories into one database. The authors present statistics about how many genes have been published in journal articles as differentially expressed at the mRNA level and how many genes have been published in journal articles as differentially expressed at the protein level in CRC studies. Nothing in Sagynaliev indicates that differential mRNA levels are not

typically reflected by differential levels of the encoded polypeptide. Instead, Sagynaliev simply notes that more differentially expressed mRNAs were reported compared to the number of reported differentially expressed proteins.

Nearly the entirety of Sagynaliev's discussion of these observations is directed to various problems with the methodology of the various studies and problems of attempting to assemble a "data warehouse" based on these studies. Of note, Sagynaliev explains that the disparate results arise at least in part due to the different studies using "heterogenous samples" of different cell sources; for example, some studies used cell lines, while others used whole tissue biopsies and still others used purified surgical specimens. Further, Sagynaliev teaches that results from one cell source (cell lines) do not allow accurate comparison for other cell sources (normal vs. cancer cells). *Sagynaliev* at pg 3072. Sagynaliev also points out that "proteomics studies [using] 2-D PAGE or 2-D DIGE have well-known technological limitations" and that 2-D PAGE analysis is "hampered by a significant variability." *Sagynaliev* at pg 3077.

Even absent these teachings of Sagynaliev, the reason for the disparity between the number of published differentially expressed mRNAs versus the number of published differentially expressed proteins is readily apparent based on the PTO's cited references of Nagaraja and Waghray, which teach the disparate sensitivities in methods for detecting nucleotides compared to methods for detecting proteins. That is, in both Nagaraja and Waghray, far fewer proteins were detectable compared to mRNAs due to the detection limitations of 2-D PAGE methods.

Furthermore, Sagynaliev provides no basis to conclude that differential mRNA levels are not typically reflected by differential levels of the encoded polypeptide. Sagynaliev reports and discusses consideration for assembling results generated from different methodologies. Sagynaliev's assembled data are not directed to the question of how differential mRNA levels influence polypeptide levels. Sagynaliev does not provide a single example in which differential mRNA levels for a particular gene were accompanied by unchanged, or oppositely differential, protein levels. Accordingly, Sagynaliev's findings cannot lead one to conclude that based on the assembled published data on CRC, differential mRNA levels do not lead to corresponding differential levels of the encoded polypeptide.

The PTO cites the studies of Nagaraja *et al.*, Waghray *et al.* and Sagynaliev *et al.* as allegedly supporting the contention that differential mRNA levels are not typically accompanied by corresponding differential protein levels. However, Nagaraja *et al.* and Sagynaliev *et al.* do not report findings contrary to Applicants asserted utility. There is not even a single example in Nagaraja *et al.* or Sagynaliev *et al.* which shows that for a particular differentially expressed mRNA, polypeptide levels were not similarly differentially expressed. The "comprehensive study" of Waghray *et al.* reports 2 instances of discernibly differentially expressed mRNAs in which the differential mRNA levels were not accompanied by similarly differential protein levels. However, the probative value of these 2 instances is questionable in view of the arbitrary time points selected for measuring dynamic mRNA and protein levels. In conclusion, the whole of the teachings of Nagaraja *et al.*, Waghray *et al.* and Sagynaliev *et al.* provide little or no evidence that would lead one skilled in the art to doubt Applicants' asserted utility.

For the reasons cited above, the references of Nagaraja *et al.*, Waghray *et al.* and Sagynaliev *et al.* provide little or no evidence to draw conclusions about the relationship between differential mRNA levels and consequent protein levels. In contrast, the references in Exhibits 3-20, which examined individually a large number of single genes or small groups of genes, provide evidence of the Applicants' position that differential mRNA levels and protein levels are correlated.

The PTO discounts Orntoft *et al.* Orntoft *et al.* examines more than just a small group of genes and is more relevant than the references cited by the PTO to whether it is well-established in the art that differential levels of mRNA encoding a particular protein generally lead to a corresponding difference in the level of the encoded protein. Nonetheless, holding Orntoft to a much higher level of scrutiny than it holds its own references, the PTO dismisses Orntoft arguing that its relevance is not clear. Applicants' submit that Orntoft *et al.* is far more relevant than any of the PTO's references and submit that it provides sound basis for supporting the conclusion that differential mRNA levels are typically accompanied by differential levels of the encoded polypeptide. The PTO cannot simply ignore the teachings of Orntoft in favor of the teachings of Nagaraja *et al.*, Waghray *et al.* and Sagynaliev *et al.*, which are far less relevant.

Orntoft *et al.* examined differences in the genetic changes that underlie invasive versus non-invasive bladder cancer. The authors examined 40 well resolved abundant known proteins, and found that "[i]n general there was a highly significant correlation ($p<0.005$) between mRNA and protein alterations. Only one gene showed disagreement between transcript alteration and protein alteration." The alterations in mRNA and protein included both increases and decreases. Clearly, a correlation in 39 of 40 genes examined supports Applicants' assertion that differential mRNA levels generally lead to corresponding differential protein levels. The PTO has not provided any basis to question any of the teachings of Orntoft.

In contrast, Applicants have shown above that the findings of Nagaraja *et al.*, Waghray *et al.* and Sagynaliev *et al.* cannot be relied upon to draw conclusions about the relationship between mRNA and protein levels. The references of Exhibits 2-13, including Orntoft *et al.*, however, provide strong evidence supporting the Applicants' position.

The Accepted Consensus in the Art is that Differential Expression of mRNA Generally Lead to Differential Expression of the Encoded Protein

The PTO argues that the state of the art clearly establishes that polypeptide levels cannot be accurately predicted from mRNA levels. However, this is not the correct technical question or the correct legal standard. Here the utility depends upon whether generally differential mRNA levels correlate with differential protein levels. More specifically in this case, utility depends upon whether given the differential expression of the PRO3566 mRNA in melanoma and esophageal tumors as compared to their normal tissue counterparts; it is more likely than not that the PRO3566 polypeptide is also differentially expressed in melanoma and esophageal tumors as compared to their normal tissue counterparts.

The additional literature references relied upon by the PTO (Lilley *et al.*, Wildsmith *et al.*, King *et al.*, Haynes *et al.*, Bork *et al.*, Madoz-Gurpide *et al.* Celis *et al.* and Lee *et al.*) are not relevant to the question of utility. None of the references report findings contrary to Applicants' asserted utility. In fact some of the references appear to support Applicants' utility arguments. The references do not refute the general rule, but at most acknowledge that there may be some exceptions to the general rule. There are no examples in the references showing that for a particular differentially expressed mRNA, polypeptide levels were not similarly differentially

expressed. Furthermore, it is worth noting that most of the citations relied upon by the PTO are in the context of DNA microarray studies. As previously discussed, Applicants are not relying on microarray data in this application. Instead, Applicants are relying on a more accurate and reliable method of assessing changes in mRNA level, namely quantitative PCR analysis. In a recent study by Kuo *et al.*, (Proteomics 5(4):894-906 (2005); previously submitted), the authors report that "[c]omparison of microarray and proteomic expression profiles showed poor correlation. Use of more reliable and sensitive analyses, such as reverse transcriptase polymerase chain reaction [RT-PCR], Western blotting and functional assays, on several genes and proteins, nonetheless, confirmed that there is indeed good correlation between mRNA and protein expression." *Kuo et al.* at Abstract (emphasis added). Thus, even if accurate, the microarray references are not relevant to the instant application which does not rely on microarray data.

In conclusion, the whole of the teachings of the references relied upon by the Examiner provide little or no evidence that would lead one skilled in the art to doubt Applicants' asserted utility.

Finally, Applicants submit herewith a copy of a declaration by Randy Scott, Ph.D. (attached as Exhibit 1). Dr. Scott is an independent expert in the field of molecular diagnostics, with over 15 years experience. He is the author of over 40 scientific publications in the fields of protein biology, gene discovery, and cancer, and is an inventor on several issued patents. His curriculum vitae is attached to the declaration. In paragraph 10 of his declaration, Dr. Scott states:

> One reason for the success and wide-spread use of the DNA microarray technique, which has led to the emergence of a new industry, is that generally there is a good correlation between mRNA levels determined by microarray analysis and expression levels of the translated protein. Although there are some exceptions on an individual gene basis, it has been a consensus in the scientific community that elevated mRNA levels are good predictors of increased abundance of the corresponding translated proteins in a particular tissue. Therefore, diagnostic markers and drug candidates can be readily and efficiently screened and identified using this technique, without the need to directly measure individual protein expression levels. *Scott Declaration* at ¶10 (emphasis added).

Applicants submit the opinion of yet another expert in the field that differential mRNA levels for a particular protein in a given tissue generally lead to corresponding differential levels of the encoded protein. Importantly, Dr. Scott also states that, contrary to the contentions of the PTO,

diagnostic markers can be identified "without the need to directly measure individual protein expression levels." This opinion is supported by Dr. Scott's extensive experience in the field, as well as the fact that an entire industry has developed around technology to assess differential mRNA expression. As stated previously, there would be little reason to study differential mRNA expression levels if those differences did not result in corresponding differential encoded protein levels.

The case law has clearly established that in considering affidavit evidence, the PTO must consider all of the evidence of record anew. *In re Rinehart,* 531 F.2d 1084, 189 USPQ 143 (C.C.P.A. 1976) and *In re Piasecki,* 745 F.2d. 1015, 226 USPQ 881 (Fed. Cir. 1985). "After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of the evidence with due consideration to persuasiveness of argument." *In re Alton,* 37 U.S.P.Q.2d 1578, 1584 (Fed. Cir. 1996)(quoting *In re Oetiker,* 977 F.2d 1443, 1445, 24 U.S.P.Q.2d 1443, 1444 (Fed. Cir. 1992)). Furthermore, the Federal Court of Appeals held in *In re Alton*, "We are aware of no reason why opinion evidence relating to a fact issue should not be considered by an examiner." *Id.* at 1583. Applicants also respectfully draw the PTO's attention to the Utility Examination Guidelines which state, "Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered." Part IIB, 66 Fed. Reg. 1098 (2001).

Utility – Conclusion

Applicants remind the PTO that the evidence supporting utility does not need to be direct evidence, nor does it need to provide an exact correlation between the submitted evidence and the asserted utility. Instead, evidence which is "reasonably" correlated with the asserted utility is sufficient. *See Fujikawa v. Wattanasin*, 93 F.3d 1559, 1565, 39 U.S.P.Q. 2d 1895 (Fed. Cir. 1996) ("a 'rigorous correlation' need not be shown in order to establish practical utility; 'reasonable correlation' suffices"); *Cross v. Iizuka*, 753 F.2d 1040, 1050, 224 U.S.P.Q. 739 (Fed. Cir. 1985) (same); *Nelson v. Bowler*, 626 F.2d 853, 857, 206 U.S.P.Q. 881 (C.C.P.A. 1980) (same). In addition, utility need only be shown to be "more likely than not true," not to a

statistical certainty. *M.P.E.P.* at § 2107.02, part VII (2004). Considering the evidence as a whole in light of the relevant standards for establishing utility, Applicants have established at least one specific, substantial, and credible utility. In view of the above, Applicants respectfully request that the PTO reconsider and withdraw the utility rejection under 35 U.S.C. §101.

Rejections under 35 U.S.C. § 112, first paragraph – Written Description

The PTO also maintains its rejection of pending Claims 6-8 and 11-17 under 35 U.S.C. § 112, first paragraph. Specifically, The PTO continues to assert that "the claims have no functional limitations ... [and that] the specification does not provide a utility or function for PRO3566." *Office Action* at 20. Also, the PTO argues that "[t]he claimed polypeptide sequences may have functions and structures which differ greatly from that of PRO3566, and therefore one of skill in the art would not be able to predictably identify the encompassed molecules as having the same functional limitations to those instantly claimed." *Office Action* at 20.

As set forth in the M.P.E.P. in § 2163, "[a]n applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention. *See Lockwood v. American Airlines, Inc.*, 107 F.3d 1565, 1572 (Fed. Cir. 1997). Also, "[p]ossession may be shown in a variety of ways including ... by describing distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention. *See,* M.P.E.P. in § 2163 (referencing *Pfaff v. Wells Elecs., Inc.*, 525 U.S. 55, 68 (1998); *Eli Lilly*, 119 F.3d at 1568; and *Amgen, Inc. v. Chugai Pharmaceutical*, 927 F.2d 1200, 1206 (Fed. Cir. 1991)

In the Office Action the PTO mischaracterizes Applicants' previous argument, stating "[a]pplicant argues that mere sequence identity (95% or 99%) bestows upon the claimed invention all of the functional limitations recited in the instant claims." Applicants incorporate their previously made arguments in support of the written description of the pending claims. Applicants are <u>not</u> relying upon mere sequence identity. Applicants submit that the written description requirement is satisfied due to the inclusion of distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention. Here, the entire amino acid sequence of SEQ ID NO:64 is fully described. That sequence in

connection with the limitation in the claims that the isolated polypeptide or a fragment thereof can be used to generate an antibody which can be used to specifically detect the polypeptide of SEQ ID NO: 64 in skin tissue or esophagus samples, is sufficient to demonstrate possession of the claimed invention. It is also worth noting that 95% and 99% variants will differ from SEQ ID NO:64 by very few amino acids. One of skill in the art would conclude that Applicants possessed at least the claimed subject based upon the combination of the very high amino acid sequence identity and the requirement that the isolated polypeptide or a fragment thereof can be used to generate an antibody which can be used to specifically detect the polypeptide of SEQ ID NO: 64 in skin or esophagus tissue samples. The combination of distinguishing identifying characteristics results in little, if any, variability in the species falling within the scope of the instant claims.

The PTO also concludes that the claims lack written description arguing that "a polypeptide sharing 95% amino acid sequence identity with SEQ ID NO:64 would be capable of being used to make antibodies not only specific to SEQ ID NO:64, but also specific to its own sequences and a great many related sequences. The PTO's conclusion is unsupported and ignores the recited limitations of the claims. For example, the claims recite that the isolated polypeptides or fragments can be used to generate an antibody which can be used to specifically detect the polypeptide of SEQ ID NO: 64 in skin tissue or esophagus samples. This limitation is sufficient combined with the recited percentage of identity to demonstrate to one skilled in the art that Applicants possessed the claimed subject matter at the time of filing the application.

In conclusion, the PTO has failed to meet its "initial burden of presenting by a preponderance of evidence why a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims." *M.P.E.P.* § 2163.04. And even if it has met this burden, Applicants submit that they have satisfied the written description requirement for the pending claims based on the actual reduction to practice of SEQ ID NO:64, by specifying a high level of amino acid sequence identity, and by describing how to make antibodies to the disclosed sequence, all of which result in a lack of substantial variability in the species falling within the scope of the instant claims. Applicants submit that this disclosure would allow one of skill in the art to "recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus."

Hence, Applicants respectfully request that the PTO reconsider and withdraw the written description rejection under 35 U.S.C. §112.

Rejections under 35 U.S.C. § 112, first paragraph – Enablement

The PTO also maintains its rejection of pending Claims 6-8 and 11-17 under 35 U.S.C. § 112, first paragraph. Specifically, the PTO asserts that because the claimed invention is not supported by either a specific or substantial asserted utility or a well established utility, one skilled in the art clearly would not know how to use the claimed invention. *Office Action* at 21.

Applicants submit that in the discussion of the 35 U.S.C. § 101 rejection above, Applicants have established a substantial, specific, and credible utility for the claimed polypeptides. To the extent that the enablement rejection is based on a lack of utility, Applicants respectfully request that the PTO reconsider and withdraw the enablement rejection under 35 U.S.C. §112.

Applicants point out that the PTO continues to make arguments that were raised in connection with now canceled claims, *e.g.*, canceled Claims 4-5. The rejections and arguments as they relate to canceled claims will not be addressed in this response.

The PTO continues to reject Claims 14-17 arguing that variant polypeptides with 95% or 99% identify are not enabled. The Examiner does make several arguments in an attempt to support the enablement rejection of pending Claims 14-17, none of which are necessary for enablement of the claims and none of which refute the fact that one of skill in the easily can make the claimed subject matter without undue experimentation. Applicants remind the Examiner that experimentation is permissible as long as it is not unduly burdensome.

The PTO continues to argue that Applicants have not specified certain regions of SEQ ID NO:64 which contain epitopes particular to an anti-PR3566 antibody. Thus, the Examiner argues that the claims are another means for claiming a polypeptide having percent identity to SEQ ID NO:64. The Examiner argues that one skilled in the art would not know how to make a protein at least 95% or 99% identical to SEQ ID NO:64 such that antibodies raised against the sequence would specifically recognize SEQ ID NO:64 and not other sequences 95% or 99% identical to SEQ ID NO:64.

The subject matter of Claims 14-17 relates to isolated polypeptides with at least 95% identity to the disclosed polypeptides wherein the isolated polypeptide or a fragment thereof can be used to generate an antibody which can be used to specifically detect the polypeptide of SEQ ID NO: 64 in skin tissue or esophagus tissue samples. The PTO has not offered any explanation of how undue experimentation is required to enable this claim.

Quite simply, specification teaches one of skill in the art to make the claimed variants. The specification teaches in detail how to make the claimed polypeptides, including variants thereof, and antibodies which specifically bind PRO3566. *See, e.g.,* ¶¶ [0283]-[0315]; [0256]-[0271]; [0361]-[0379]; and Examples 6-10 (¶¶ [0453]-[0499]). In addition, the specification discloses that antibodies to claimed polypeptides can be used in diagnostic assays to detect the expression of PRO3566 in specific types of tissue. *See e.g., Specification* at [0407]. Thus, there is significant guidance on how to make and use the claimed polypeptides without undue experimentation. Applicants again note that the production of polypeptides, polypeptide variants, and specific antibodies is a predictable and well established aspect of the biological sciences. *See, e.g., In re Wands*, 858 F.2d 731, 8 U.S.P.Q. 2d 1400 (Fed. Cir. 1988) (reversing the Board's decision of non-enablement and holding that as of 1980, undue experimentation was not required to make high-affinity monoclonal antibodies to a target peptide); *Sutcliffe et al.,* Science (1983) 219:660-666 at 661-662 (teaching that "by following simple rules, one can in general select peptides that will elicit antibodies reactive with intact proteins") (previously submitted).

Furthermore, enablement does not require disclosure of the particular portion or region of the polypeptide that results in an observed or claimed activity or the portion that is recognized by a biological molecule such as an antibody. For example, in Example 14 of the written description guidelines, the specific region of a polypeptide required for the molecule to have its activity is not required. Presumably, this is because the skilled artisan could make a variant without knowing which portion of the sequence causes the activity, and easily test (without undue experimentation) to verify that a variant has the activity. For similar reasons, it is not required in this case to specify particular regions of SEQ ID NO:64 which contain epitopes particular to an anti-PRO3566 antibody. One of skill in the art can easily make a 95% or 99%

variant and test to determine that the variant can generate antibodies that specifically detect the amino acid of SEQ ID NO:64 is esophageal or skin tissue samples.

In conclusion, the PTO's rejection based on lack of utility has been addressed above, and the PTO has otherwise failed to meet its burden to establish a reasonable basis to question the enablement provided for the claimed invention. Given the skill in the art and the disclosure of how to make and use the claimed polypeptides, Applicants request that the PTO reconsider and withdraw its rejection under 35 U.S.C. § 112, first paragraph.

CONCLUSION

In view of the above, Applicants respectfully maintain that claims are patentable and request that they be passed to issue. Applicants invite the Examiner to call the undersigned if any remaining issues may be resolved by telephone.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 1/16/2007

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